at the synapse. Regardless of the final explanation for the unexpected results with LAT, genetics has served its purpose in calling attention to a molecule that is not a usual suspect at the synapse.

How, in the end, should we think about the learning phenotype of *latheo* mutants? Is it a result of defective mushroom body development, impaired synaptic plasticity, or both? A satisfying answer must await the identification of mutants that separate the roles of *lat* at the chromosome and the synapse, or the creation of conditional mutants that rescue only the developmental defects. In the meantime, these two papers in *Neuron* may inspire neurobiologists to head to the library for a refresher course on DNA replication.

Aaron DiAntonio
Department of Molecular and Cell Biology
University of California, Berkeley
Berkeley, California 94720

Selected Reading

Boynton, S., and Tully, T. (1992). Genetics *131*, 655–672.

Dubnau, J., and Tully, T. (1998). Annu. Rev. Neurosci. *21*, 407–444.

Gatti, M., and Baker, B.S. (1989). Genes Dev. *3*, 438–453.

Gossen, M., Pak, D.T.S., Hansen, S.K., Acharya, J.K., and Botchan, M.R. (1995). Science *270*, 1674–1677.

Heisenberg, M., Borst, A., Wagner, S., and Byers, D. (1985). J. Neurogenet. *2*, 1–30.

Landis, G., Kelley, R., Spradling, A.C., and Tower, J. (1997). Proc. Natl. Acad. Sci. USA *94*, 3888–3892.

Nusslein-Volhard, C., and Weischaus, E. (1980). Nature *287*, 795–801.

Pinto, S., Quintana, D.G., Smith, P., Mihalek, R.M., Hou, Z.-H., Boynton, S., Jones, C.J., Hendricks, M., Velinzon, K., Wohlschlegel, J.A., et al. (1999). Neuron *23*, this issue, 45–54.

Rohrbough, J., Pinto, S., Mihalek, R.M., Tully, T., and Broadie, K. (1999). Neuron *23*, this issue, 55–70.

Rowles, A., and Blow, J.J. (1997). Curr. Opin. Genet. Dev. *7*, 152–157.

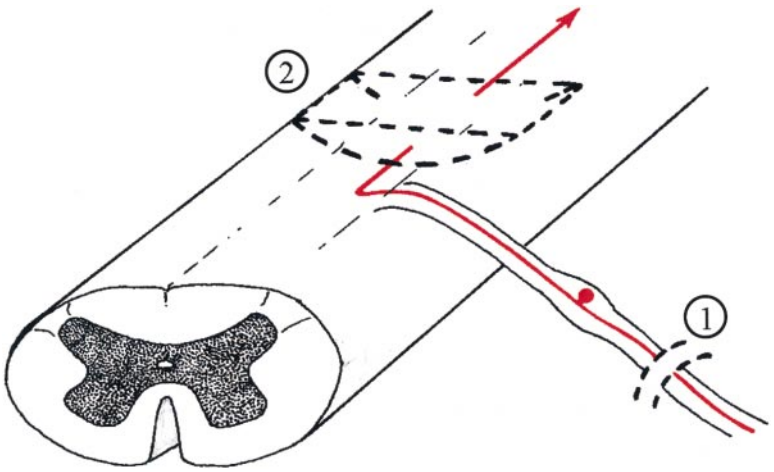
Zhong, Y., and Wu, C.F. (1991). Science *251*, 198–201.

The Benefits of Adding Insult to Injury

A persistent, central question in neurobiology is, why do the axons of the PNS regenerate after injury while CNS axons do not? Several factors are known to prevent CNS regeneration: glial scarring (which presents both a physical barrier and inhibitors to regeneration), myelin-specific inhibitors, and possibly the loss of general growth capacity by adult CNS axons. In contrast, the PNS regenerates largely because of the environmental changes following injury. Myelin debris is cleared and Schwann cells dedifferentiate, downregulating expression of myelin proteins and thus becoming permissive for regeneration. Not surprisingly, a number of studies, going back over a decade, demonstrate that CNS axons readily grow in vivo into an implanted graft of PNS tissue (Richardson et al., 1980; David and Aguayo, 1981).

An environment favorable to regeneration, however, is not all that is needed. Further work showed that a primary lesion in the peripheral branch of the dorsal root ganglion (DRG), followed by a second lesion in the spinal branch of the same nerve at the same time or weeks later, results in more extensive growth into peripheral nerve grafts situated in the dorsal columns. The conditioning peripheral nerve lesion somehow encourages growth for the majority of lesioned spinal axons to the most distal edge of the graft but still not into the host CNS tissue beyond. In spite of the conditioning lesion effect, it appears that a glial scar at the graft–host border, along with myelin-specific inhibitors, ultimately halts axonal growth (Richardson and Issa, 1984; Oudega et al., 1994). What is notable about these animal experiments is that the CNS axons grow better if their peripheral branch has been previously cut. Even a conditioning lesion in the peripheral branch of a DRG, followed by a second peripheral branch lesion of the same nerve rather than a central lesion, leads to more rapid regeneration (McQuarrie and Grafstein, 1973). Together, these experiments suggest a strong environmental influence on regeneration, but they also point to some intrinsic properties of the neuron that must affect the extent of regeneration. A conditioning lesion in the peripheral branch of the sciatic nerve induces an intrinsic change in the neuron that allows either the peripheral or central branch to subsequently grow better after injury.

In a study reported in this issue of *Neuron*, Neumann and Woolf (1999) extend these observations by demonstrating that transected dorsal column axons regenerate when a conditioning lesion is first created in the peripheral branch of the sciatic nerve. What makes this study important, and different, is not the substantial distance traversed by the regenerating dorsal column axons, nor the possibly greater number of regenerating axons compared to other studies, but rather that no peripheral nerve graft was used. Regeneration occurred into what is, by all previous criteria, the highly nonpermissive environment of the damaged spinal cord (see figure). After corticospinal tract lesions by Schwab and colleagues, regeneration also occurred over relatively long distances, but these studies employed the monoclonal antibody IN-1, which neutralizes some of the myelin-specific inhibitors of regeneration (Bregman et al., 1995). In the study reported here, regeneration occurred without blocking myelin or glial scar inhibitors. Transecting the dorsal column and simultaneously lesioning the peripheral nerve results in extensive regeneration into the lesion site but not beyond. Significantly, however, Neumann and Woolf (1999) show that when the conditioning lesion in the peripheral nerve is performed 1 week before the dorsal column transection, about 50% of the animals show axon regrowth around the site of injury and into the gray matter surrounding the central canal, with little or no growth into the lesion site itself. Furthermore, these axons continue to grow both caudally and rostrally from the lesion site. In the remaining half of the prelesioned animals, axons did grow into the lesion site, continuing on through the lesion and beyond, to grow mostly



Sites of Preconditioning Peripheral Branch Lesion and Subsequent Central Branch Lesion in Dorsal Root Ganglion Nerves

Preconditioning lesions were created in the sciatic nerve (L4–L6) (1) either simultaneously with, or 1 or 2 weeks prior to, a dorsal column transection at T6–T7 (2). Regeneration occurred through the dorsal column lesion site if the preconditioning lesion preceded the dorsal column lesion by 1 or 2 weeks; regrowth occurred only into the lesion if the preconditioning lesion was inflicted at the same time; no regeneration occurred without a peripheral lesion. The dashed line shows the sites of lesions.

through host gray matter but with considerable regeneration also observed in white matter. A 2-week conditioning lesion resulted in slightly less effective dorsal column regeneration than a 1-week conditioning lesion. Without the peripheral nerve conditioning lesion, dorsal column axons did not regenerate. Finally, in culture, neurite growth from DRG explants was longer following an in vivo conditioning lesion than without one.

So, what is responsible for this regrowth? How can a lesion in the peripheral nervous system affect the regeneration capabilities of the CNS branch of the same nerve? The study by Neumann and Woolf (1999), along with previous studies, suggests that the growth capacity of the dorsal column axons is clearly improved by a preconditioning lesion. We do not know, however, the molecular mechanism underlying this improved growth capacity, nor do we know why these axons are not stopped by inhibitory molecules within the glial scar and myelin. One possibility is that growth commences before the glial scar can form. Alternatively, the glial scar might form differently after a conditioning lesion. Since inhibitors in myelin, whether soluble or membrane associated, are exposed/secreted in response to damage regardless of whether there is a lesion to the peripheral nerve, regrowing central axons will confront them in both cases. However, a peripheral nerve lesion might alter the response of regrowing dorsal column axons to myelin inhibitors and inhibitors in general. For example, peripheral lesions may lead to the downregulation of the receptors for these inhibitors on the central regenerating growth cone. Alternatively, inhibitor receptors may still be expressed after a peripheral lesion, but they may no longer signal inhibition. Three recent observations make this latter interpretation compelling. First, like many immature neurons, growth of embryonic and neonatal DRG neurons is not inhibited by myelin (Shewan et al., 1995). For one myelin-specific inhibitor, myelin-associated glycoprotein (MAG), the switch in response from promotion to inhibition occurs sharply at postnatal day 3/4 (DeBellard et al., 1996). Second, inhibition of axonal regeneration by MAG and myelin in culture has been shown to be overcome by elevated neuronal cAMP levels. Conversely, the growth-promoting effect of MAG or myelin on young DRG neurons is blocked by inhibitors of protein kinase A (Cai et al., 1999). Third, the repulsion of growth cones by a soluble form of MAG, and by a number of other inhibitory molecules, can be switched to attraction by elevating cAMP (Song et al., 1998). Together, these important results suggest that the neuronal response to myelin inhibitors (and, although never tested, possibly also to inhibitors in the glial scar) is dictated by the neuron's endogenous levels of cAMP. Therefore, it is quite likely that transection of the peripheral nerve branch of DRG neurons results in an increase in endogenous levels of cAMP, perhaps reaching levels found in young animals. This may neutralize the effect of myelin inhibitors or effectively switch the response of axons to myelin inhibitors to promotion, thereby allowing them to grow through white matter. Therefore, the combined effect of a conditioning lesion on both increased growth capacity and the ability to grow through inhibitors could account for the pattern of regrowth of these dorsal spinal axons in the damaged spinal cord.

If cAMP does play a major role in this improved growth through damaged spinal tissue, the next obvious questions are (1) what is activated or inactivated to achieve this effect and (2) is the response dependent on protein synthesis? Because growth is better after a 1 week conditioning lesion than when the two lesions are created at the same time, time-dependent changes must occur in the damaged spinal column axon to allow it to regrow. Consistent with this prediction, Smith and Skene (1997) show that after axotomy, adult DRG axons grow differently, becoming more elongated rather than branched, and that this transition requires ongoing transcription for a limited period after injury. An obvious candidate molecule to give improved growth is the growth-associated protein GAP43, which is upregulated in spinal axons after a conditioning lesion. However, preliminary results suggest that there is no improved regeneration in transgenic mice overexpressing GAP43 (Neumann and Woolf, 1999).

At this stage of incomplete understanding, can we suggest strategies for improving spinal cord regeneration in humans? Clearly, for both ethical and practical reasons, a conditioning peripheral nerve injury cannot be inflicted in humans to encourage growth of spinal axons. The peripheral lesion would have to be created before the spinal cord injury itself, an obviously absurd

approach. The solution, instead, is to work out what is happening at the molecular level. How can this effect be induced or mimicked in axons without a conditioning lesion? Can it be induced in neurons that do not have a peripheral nerve branch? Why did the dorsal column axons stop growing before they reached their original target? Perhaps the initial signal induced by the conditioning lesion had subsided. If so, would a second lesion encourage them to start growing again? Answers to these questions could lead to a rational, molecular approach to encouraging CNS axons to regrow after injury. The next problem, of course, is then getting them to their correct destination.

Marie T. Filbin
Department of Biological Sciences
Hunter College of the City University of New York
New York, New York 10021

Selected Reading

Bregman, B.S., Kunkel-Bagden, E., Schnell, L., Dai, H.N., Gao, D., and Schwab, M.E. (1995). Nature *378*, 498–501.

Cai, D., Shen, Y., De Bellard, M.E., Tang, S., and Filbin, M.T. (1999). Neuron *22*, 89–101.

David, S., and Aguayo, A.J. (1981). Science *214*, 931–933.

DeBellard, M.E., Tang, S., Mukhopadhyay, G., Shen, Y., and Filbin, M.T. (1996). Mol. Cell. Neurosci. *7*, 89–101.

McQuarrie, I.G., and Grafstein, B. (1973). Exp. Neurol. *29*, 53–55.

Neumann, S., and Woolf, C.J. (1999). Neuron *23*, this issue, 83–91.

Oudega, M., Varon, S., and Hagg, T. (1994). Exp. Neurol. *129*, 194–206.

Richardson, P.M., and Issa, V.M.K. (1984). Nature *309*, 791–793.

Richardson, P.M., McGuinness, U.M., and Aguayo, A.J. (1980). Nature *284*, 264–265.

Shewan, D., Berry, M., and Cohen, J. (1995). J. Neurosci. *15*, 2057–2062.

Smith, D.S., and Skene, J.H.P. (1997). J. Neurosci. *17*, 646–658.

Song, H., Ming, G., Lehmann, M., McKerracher, L., Tessier-Lavigne, M., and Poo, M.-M. (1998). Science *281*, 1515–1518.

α Neurotoxins and Their Relatives: Foes and Friends?

In 1963, C. C. Chang and C. Y. Lee initiated a new era in the study of neurotransmitter receptors by showing that a small protein toxin derived from the Taiwanese snake *Bungarus multicinctus* bound tightly and specifically to the nicotinic acetylcholine receptor (nAChR) at the vertebrate neuromuscular junction (Chang and Lee, 1963). At the time of the discovery of α-bungarotoxin (αBtx) and its cobratoxin relatives, the nAChR, although physiologically and pharmacologically well defined, was a molecular enigma. Even the question of whether it was a protein was disputed. The advent of αBtx and related α neurotoxins completely transformed the field. Their extremely high affinity and long off-times (making them essentially irreversible antagonists), their extraordinary specificity, and the ease with which radioactive or fluorescent labels are incorporated made the α neurotoxins indispensable experimental tools for the study of nAChRs. Their use immediately led to the identification, purification, and localization of these receptors in muscle and in the *Torpedo* electric organ, and made the nAChR the paradigmatic neurotransmitter receptor for the next 20 years.

αBtx is an effective toxin because it inhibits the binding of acetylcholine to the AChR, producing neuromuscular blockade and quick death. This efficient strategy is used by a large number of poisonous snakes in the Elapidae family (cobras, kraits, mambas, and tiger snakes, among others), whose venoms collectively contain over 100 toxins with sequence homology to αBtx. All of these toxins share a common structure consisting of a β sheet core that is tightly cross-linked by four invariant disulfide bonds. Three flexible loops extend from the core to bind the receptor (Love and Stroud, 1986). Most of the toxins in the family bind the nAChR, but some bind other molecules, including muscarinic AChRs and acetylcholinesterase.

The idea that potent toxins have evolved to recognize physiological binding sites for a natural ligand such as acetylcholine is a familiar one. Recent studies by Miwa et al. (1999), however, reported in this issue of *Neuron*, give this concept a new twist. They suggest that the α neurotoxins not only mimic but also may be evolutionarily related to an endogenous ligand for the AChR, one that enhances rather than inhibits the action of acetylcholine. This new finding not only sheds light on the evolutionary origins of this family of toxins, but also poses the question of how the endogenous peptide might function physiologically to modulate the AChR.

The beneficent new relative of the family of elapid neurotoxins is lynx1, a small (11 kDa) protein that was identified in the course of a search for developmentally regulated genes in the cerebellum. A database search using the amino acid sequence encoded by the *lynx1* gene immediately revealed homology with αBtx and with the Ly-6 family, a related group of proteins that are found on the surface of mouse lymphocytes (Gumley et al., 1995). The Ly-6 proteins have tertiary structures that are similar to αBtx and are apparently attached to the surface membrane through a glycolipid anchor, where they participate in cell–cell and cell–substrate interactions. lynx1 shares with both αBtx and Ly-6 the highly conserved motif of eight cysteines, and models show that its predicted three-dimensional structure closely resembles the experimentally determined structures of αBtx and CD59, a member of the Ly-6 family. Moreover, the exon–intron boundaries of all three proteins are the same—a strong indication of a common evolutionary origin.

What distinguishes lynx1, and makes it of unusual interest, is evidence suggesting that it has a functional relationship to AChRs in the nervous system. lynx1 is highly expressed in the brain, where it is associated with neurons in the cortex, in the hippocampus, and in the cerebellum. Interestingly, each of the sites of lynx1 localization in the brain is also a site at which the α7 neuronal AChR is expressed. The α7 AChR, a homooligomeric